REMARKS

Claims 1, 2, 15, 16 and 18-24 are pending in the subject application. By this Amendment, applicants have amended claim 15. In view of the arguments below, applicants maintain that the Examiner's rejections have been overcome, and respectfully request that they be withdrawn.

Objection to the Specification

The Examiner objected to the specification as allegedly failing to comply with the requirements of 37 C.F.R. §1.821-§1.825 and encloses a Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures. The Notice To Comply, a copy of which is attached hereto as **Exhibit A**, states that applicants are required to provide SEQ ID NOs for sequences listed in the specification. Specifically, the Notice requires SEQ ID NOs on pages 24, 25 and 26, and Figure 14.

In response, applicants note that these amendments to the specification regarding SEQ ID NOs. have already been made in a November 19, 2002 Amendment responding to a September 19, 2002 Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures. For the Examiner's convenience, applicants attach hereto a copy of the November 19, 2002 Amendment, including a paper copy of the Sequence Listing, and a Statement Pursuant to

37 C.F.R. §1.821, attached hereto as **Exhibit B**. Accordingly, applicants maintain that the subject application complies with the requirements of 37 C.F.R. §1.821-§1.825.

Provisional Rejection Under 35 U.S.C. §101, Double Patenting

The Examiner maintained the provisional rejection of claims 1-16 and 18-24 under 35 U.S.C. §101 as claiming the same invention as that of claims 1-24 of co-pending U.S. Application No. 10/712,642.

In response, applicants note that claims 1-24 were canceled and new claims 27-40 were added via a November 12, 2003 Amendment. Accordingly, the Examiner's provisional rejection is moot.

Rejection under 35 U.S.C. §102(b)

The Examiner rejected claim 15 under 35 U.S.C. §102(b) as allegedly anticipated by Connelly et al.

In response, applicants respectfully traverse.

Under 35 U.S.C. §102(b), a person shall be entitled to a patent unless the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States. According to MPEP

§2131.01, "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

Applicants note that Connelly et al. does not teach each and every element of amended claim 15. Claim 15 provides an antisense oligonucleotide that specifically hybridizes to a nucleic acid encoding a human *Ku70 subunit* of DNA-PK. Applicants note that Connelly et al. teach an anti-sense RT-PCR primer which is capable of hybridizing to a human *catalytic subunit* of DNA-PK. Connelly et al. does not disclose antisense oligonucleotides capable of specifically hybridizing to human DNA-PK Ku70 subunit-encoding nucleic acid. Accordingly, applicants maintain that amended claim 15 is not anticipated by Connelly et al.

In view of these remarks, applicants maintain that amended claim 15 satisfies the requirements of 35 U.S.C. §102(b), and request that the rejection be withdrawn.

Rejection Under 35 U.S.C. §103(a)

The Examiner rejected claims 1, 2, 15, 16 and 18-24 under 35 U.S.C. §103(a) as allegedly unpatentable over Connelly et al. as applied to claim 15 in the 35 U.S.C. §102(b) rejection, in view of Milner et al., Tanigushi et al. and Au-Young et al.

In response to the Examiner's rejection, applicants respectfully traverse, and maintain that the Examiner has failed to establish a *prima facie* case of obviousness.

To establish a *prima facie* case of obviousness, the Examiner must demonstrate three criteria with respect to each claim. First, the cited references, when combined, teach or suggest every element of the claim. Second, one of ordinary skill would have been motivated to combine the teachings of the cited references at the time of the invention. And third, there would have been a reasonable expectation that the claimed invention would succeed.

In light of these requirements, applicants maintain that the cited references fail to support a *prima facie* case of obviousness for independent claims 1 and 15 and dependent claims 2, 16 and 18-24.

The rejected claims provide antisense oligonucleotides which specifically hybridize to nucleic acids encoding human DNA-PK Ku70 subunits and methods for increasing the susceptibility of a cell to DNA-damaging agents. The method of claim 1 and the antisense oligonucleotide of claim 15 are, at least in part, based on applicants' surprising discovery of Ku70's role in DNA double-stranded breaks repair. As detailed above, Connelly et al. teach an antisense molecule that hybridizes to a nucleic acid encoding the human DNA-PK catalytic subunit. As the

Examiner states in the December 23, 2004 Final Office Action, Reeves et al. is relied upon for teaching the nucleic acid sequence encoding the DNA-PK Ku70 subunit, and Milner et al. teaches methods of designing and testing antisense oligonucleotides. As the Examiner also states in the December 23, 2004 Final Office Action, Tanigushi et al. teach antisense oligonucleotide which hybridize to mouse DNA-PK subunits, and Au Young et al. teach pharmaceutical compositions comprising antisense oligonucleotides.

In response to the rejection of claims 1 and 2, applicants maintain that as stated above, none of the references teach a method for increasing the susceptibility of a cell to DNA-damaging agents, comprising introducing into the cell *in vitro* an antisense oligonucleotide that specifically hybridizes to a nucleic acid encoding a human DNA-dependent protein kinase subunit so as to prevent expression of the human DNA-dependent protein kinase subunit. None of the cited references teaches the introduction of any antisense oligonucleotide into any cell for the purpose of increasing the susceptibility of the cell to DNA-damaging agents. Therefore, applicants maintain that the cited references in combination fail to teach each and every element of claims 1 and 2.

In response to the rejection of claims 15, 16 and 18-24, applicants maintain that as also stated above, none of the cited references teaches an antisense oligonucleotide that specifically hybridizes to a nucleic acid encoding a human Ku70 subunit of DNA-PK, as provided by amended claim 15 and as

recited in dependent claims 16 and 18-24. Therefore, applicants maintain that the cited references in combination fail to teach each and every element of claims 15, 16 and 18-24.

For the reasons above, the cited references combined fail to teach the elements of the claimed antisense oligonucleotide or the elements of the claimed methods. Absent such teaching, there could not have been a motive to combine or a reasonable expectation of success.

In view of the above remarks, applicants maintain that the Examiner has failed to set forth a *prima facie* case of obviousness, and that accordingly, claims 1, 2, 15, 16 and 18-24 satisfy the requirements of 35 U.S.C. §103(a).

Conclusion

For the reasons set forth above, applicants respectfully request that the Examiner reconsider and withdraw the rejections, and solicit allowance of pending claims 1, 2, 15, 16 and 18-24.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee, other than the $60.00 extension fee, is deemed necessary in connection with the filing of this Amendment. However, if any additional fee is required, authorization is given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:
Commissioner for Patents,
P.O. Box 1450
Alexandria, VA 22313-1450.

4/27/01

Alan J. Morrison Date
Reg. No. 37,399

John P. White
Registration No. 28,678
Alan J. Morrison
Registration No. 37,399
Attorneys for Applicants
Cooper & Dunham LLP
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New York, New York 10036
(212) 278-0400

Application No.: 09/750,440

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s):

☒ 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.

☐ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c).

☐ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).

☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked -up "Raw Sequence Listing."

☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).

☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable from of the "Sequence Listing" as required by 37 C.F.R. 1.821(e).

☒ 7. Other: Please Provide SeQ ID Nos For SeQuences Listed in Spec (pp 24, 25, 26 & Fig 14).

Applicant Must Provide:

☒ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing".

☒ An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification.

☒ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

For questions regarding compliance to these requirements, please contact:

For Rules Interpretation, call (703) 308-4216
For CRF Submission Help, call (703) 308-4212
PatentIn Software Program Support (SIRA)
Technical Assistance.....703-287-0200
To Purchase PatentIn Software.....703-306-2600

PLEASE RETURN A COPY OF THIS NOTICE WITH YOUR RESPONSE

Dkt. 55672-A-PCT-US/JPW/AJM/MVM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Gloria C. Li et al.

Serial No.: 09/750,410

Filed : December 28, 2000

For : USES OF DNA-PK

1185 Avenue of the Americas
New York, New York 10036
November 19, 2002

U.S. Patent and Trademark Office
BOX: Sequence
P.O. Box 2327
Arlington, VA 22202

SIR:

AMENDMENT IN RESPONSE TO SEPTEMBER 19, 2002 NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

This Amendment is submitted in response to September 19, 2002 Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures issued by the United States Patent and Trademark Office in connection with the above-identified application. A copy of the Notice is attached hereto as Exhibit A. The Notice provides a two-month period for filing a response. Therefore, a response to the September 19, 2002 Notice is due November 19, 2002. Accordingly, this Amendment is being timely filed.

Please amend the subject application as follows:

In the specification:

Following the section entitled "Abstract of the Disclosure" and before the Figures, please add the paper copy of the "Sequence Listing", attached hereto as Exhibit B.

In addition, please amend the specification under the provisions of 37 C.F.R. §1.121(b)(1) as follows.

On page 12, lines 10-23, please delete the paragraph which begins "Figure 14" and insert the following paragraph:

Figure 14

Nucleotide sequences of Vβ8Dβ2.1Jβ2.6 junctions from the thymus of a 4 week old *Ku70-/-* mouse (SEQ ID NOS: 1-23). Products corresponding to Vβ8.1, Vβ8.2 or Vβ8.3 rearrangement with Jβ2.6 were cloned and sequenced. TCR Vβ8-Jβ2 joints were amplified by PCR (20, 27, 28) as described (see Fig. 3B). PCR cycling conditions were 94°C for 45", 68°C for 30", and 72°C for 30" (30 cycles). The band corresponding to Vβ8-Jβ2.6 was purified, reamplified for 20 cycles and then subcloned into the pCRII vector (Invitrogen). DNA was extracted from individual colonies and sequenced using the universal T7 and M13 reverse primers. Germline sequences are written in bold case, 'N' and 'P' denote nucleotides not present in the germline sequences.

On page 24, lines 25-32, please delete the text which begins "The genotype of the mice was first determined . . ." and insert the following text:

The genotype of the mice was first determined by tail PCR analysis which distinguishes endogenous from the targeted *Ku70* allele, and subsequently confirmed by Southern blot analysis. The PCR reaction contained 1 μg genomic DNA; 0.6 μM (each) of primers HO-2: GGGCCAGCTCATTCCTCCACTCATG (SEQ ID NO: 24), HO-3: CCTACAGTGTACCCGGACCTATGCC (SEQ ID NO: 25) and HO-4: CGGAACAGGACTG-GTGGTTGAGCC (SEQ ID NO: 26); 0.2 mM (each) dNTP;

1.5 mM $MgCl_2$ and 2.5 U of Taq polymerase. Cycling conditions were 94°C for 1 min,

On page 25, lines 8-20, please delete the paragraph which begins "To confirm that the disruption of *Ku70* . . ." and insert the following paragraph:

To confirm that the disruption of *Ku70* produces a null mutation, Ku70 protein expression was measured by Western blotting using polyclonal antisera against intact mouse Ku70. The lack of Ku70 was also verified by a Ku-DNA-end binding assay (gel mobility shift analysis). Cell extracts were prepared and gel mobility shift assays were performed as described (22). Equal amounts of cellular protein (50 μg) from *Ku70+/+* (WT), *Ku70+/-*, and *Ku70-/-* mouse embryo fibroblasts were incubated with a ^{32}P-labeled double-stranded oligonucleotide, 5'-GGGCCAAGAATCTTCCAGCAGTTTCGGG-3' (SEQ ID NO: 27). The protein-bound and free oligonucleotides were electrophoretically separated on a 4.5% native polyacrylamide gel. Gel slabs are dried and autoradiographed with Kodak X-Omat film.

On page 27, lines 1-23, please delete the paragraph which begins "To determine whether a null mutation . . ." and insert the following paragraph:

To determine whether a null mutation in *Ku70* affects the recombination of antigen-receptor genes in T and B lymphocytes *in vivo*, we measured the immunoglobulin and T-cell antigen receptor (TCR) rearrangements by PCR. DNA from bone marrow was amplified with primers specific to immunoglobulin D-J_H and V-DJ_H rearrangements, and DNA from thymus was amplified with primers that detect V-DJ_β and D_δ-J_δ-rearrangement (20, 25-28).

Oligonucleotides for probes and PCR primers specific to TCR Vβ-Jβ rearrangements and immunoglobulin D-J_H and V-DJ_H rearrangements are as follows. For TCRβ Vβ8-Jβ2 rearrangements (28): Vβ8.1: 5'-GAGGAAAGGT-GACATTGAGC-3' (SEQ ID NO: 28), Jβ2.6: 5'-GCCTGGTGCCGGGACCGAAGTA-3' (SEQ ID NO: 29), Vβ8 probe: 5'-GGGCTG AGGCTG ATCCATTA-3' (SEQ ID NO: 30). For $D_{\delta 2}$-$J_{\delta 1}$ rearrangement (20, 27): DR6: 5'-TGGCTTGACATGCAGAAAACACCTG-3' (SEQ ID NO: 31), DR53: 5'-TGAATTCCACAG-TCACTTGGCTTC-3' (SEQ ID NO: 32), and DR2 probe: 5'-GACACGTGATACAAAGCCCAGGGAA-3' (SEQ ID NO: 33). For immunoglobulin D-J_H and V-DJ_H rearrangements (26): 5'D: 5'-GTCAAGGGATCTACTACTGTG-3' (SEQ ID NO: 34), V7183: 5'-GAGAGAATTCAGAGACAATC-CCAAGAACACCCTG-3' (SEQ ID NO: 35), VJ558L: 5'-GAGAGAATTCTCCTCCAGCACAG-CCTACATG-3' (SEQ ID NO: 36), J2: 5'-GAGAGAATTCGGCTCCCAATGACCCTTTCTG-3' (SEQ ID NO: 37), 5'IVS: 5'-GTAAGAATGGCCTCTCCAGGT-3' (SEQ ID NO: 38), 3'-IVS: 5'-GACTCAATCACTAAGACA-GCT-3' (SEQ ID NO: 39), and probe: a 6 kb EcoR I fragment covering the J region of mouse IgM.

On page 62, lines 23-34, please delete the text which begins "The genotypes of the mice were first determined . . ." and insert the following text:

The genotypes of the mice were first determined by tail PCR analysis which distinguishes endogenous from the targeted Ku70 allele, and subsequently confirmed by Southern blot analysis. The PCR reaction contained 1 mg genomic DNA; 0.6 mM (each) of primers HO-2: GGGCCAGCTCATTCCTCCACTCATG (SEQ ID NO: 40), HO-3: CCTACAGTGTACCCGGACCTATGCC (SEQ ID NO: 25) and HO-4: CGGAACAGGACTGGTGGTTGAGCC (SEQ ID NO: 41); 0.2 mM (each) dNTP; 1.5 mM $MgCl_2$ and 2.5 U of Taq polymerase. Cycling conditions were 94°C for 1 min, 64°C for 1 min, 72°C for 1 min (30 cycles), followed by an extension at 72°C for 10 min. Primers HO-2 and HO-4 give a product of the targeted

On page 87, lines 17-25, please delete the paragraph which begins "The genotype of the mice was determined by PCR . . ." and insert the following paragraph:

> The genotype of the mice was determined by PCR which distinguishes endogenous from the targeted DNA-PKcs allele. PCR reaction contains 1 μg genomic DNA; 0.6 μM (each) of primers MD-20: TATCCGGAAGTCGCTTAGCA-TTG (SEQ ID NO: 42); MD-21: AAGACGGTTGAAGTCAGAAGTCC (SEQ ID NO: 43); and POL-8: TTCACATACACC-TTGTCTCCGACG (SEQ ID NO: 44); 0.2 mM(each) dNTP; 1.5 mM $MgCl_2$ and 2.5U of Taq polymerase. Primers MD-20 and MD-21 give a product of wild type allele that is 264 bp; primers MD-20 and Pol-8 yield a product of the targeted allele that is 360 bp.

On page 88, lines 7-19, please delete the paragraph which begins "For RT-PCR assay, total RNA was prepared . . ." and insert the following paragraph:

> For RT-PCR assay, total RNA was prepared from SV40 transformed lung fibroblast cells using Qiagen RNeasy kit (Qiagen Inc., Santa Clarita, CA). After digestion of contaminated genomic DNA by DNase I (Ambion, Austin TX), cDNA synthesis was carried out with the Superscript preamplification system (Gibco BRL, Gaithersburg, MD) according to the included protocol. PCR primers used for RT-PCR were MD-3: ATCAGAAGGTCTAAGGCTGGAAT (SEQ ID NO: 45), MD-5: CGTACGGTGTTGGCTACTGC (SEQ ID NO: 46) for amplification between exon 1 and 4 of DNA-PKcs , MD-28: CACTGAGGGCTT-TCCGCTCTTGT (SEQ ID NO: 47), MD-29: GCTCTTGTGCACGAATGTTGTAG (SEQ ID NO: 48) for PI-3 kinase domain, and GA-5: AGAAGACTGTGGATGGCCCC (SEQ ID NO: 49), GA-3: AGGTCCACCACCC-TGTTGC (SEQ ID NO: 50) for control GAPDH amplification.

On page 89, line 22 - page 90, line 12, please delete the paragraph which begins "T cell antigen receptor (TCR) . . ." and insert the following paragraph:

T cell antigen receptor (TCR) and immunoglobulin recombination in T and B lymphocytes were measured by amplifying rearranged DNA fragments using PCR. Genomic DNAs were isolated from thymus, spleen and bone marrow (BM) from 4-to 9-week-old DNA-PKcs heterozygous (+/-), homozygous (-/-) mice and SCID mice. Oligonucleotides for PCR primers and probes are as follow. For TCR_{β} $V_{\beta}8$-$J_{\beta}2$ rearrangement (16), $V_{\beta}8.1$: GAGGAAAGGTGACATTGAGC (SEQ ID NO: 51), $J_{\beta}2.6$: GCCTGGTGCCGGGACCGAAGTA (SEQ ID NO: 29), and $V_{\beta}8$ probe: GGGCTGAGGCTGATCCATTA (SEQ ID NO: 52). For TCR_{δ} $D_{\delta}2$-$J_{\delta}1$ rearrangement, DR6: TGGCTTGACATGCAGAAAACACCTG (SEQ ID NO: 31), DR53: TGAATTCCACAGTCACTTGGGTTC (SEQ ID NO: 53) and DR2 probe: GACACGTGATACAAAGCCCAGGGAA (SEQ ID NO: 33). For TCR_{δ} $D_{\delta}2$-$J_{\delta}1$ signal joint (19), DR21: GTCATATCTTGTCCAGTCAACTTCC (SEQ ID NO: 54), DR162: GATGAGCCAGCTGGATGAGTAACAC (SEQ ID NO: 55), and DR161 probe: GCCCTCTAGCCATGACA TCAGAGC (SEQ ID NO: 56). For immunoglobulin $V_{H}7183$-$J_{H}4$ rearrangement(19), DR214: CGCGAAGCTTCGT GGAGTCTGGGGGA (SEQ ID NO: 57), DR217: GGGGAATTCCTGAGGAGACGGTGACT (SEQ ID NO: 58), and DR218 probe: ACCCCAGTAGTCCATAGCATAGTAAT (SEQ ID NO: 59). For control GAPDH amplification, same primers were used as RT-PCR experiment. Probe DNA for mouse GAPDH was purchased from Ambion Inc.(Cat.#7330, Austin TX). Amplified PCR products were resolved on 2% of agarose gel in 0.5x TBE, and transferred to Hybond N+ nylon membrane. Using radiolabeled oligonucleotide or DNA probes, PCR products were hybridized and visualized by autoradiography.

REMARKS

The September 19, 2002 Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures states that the nucleotide and/or amino acid sequence disclosure contained in this application clearly fails to comply with the requirements of 37 C.F.R. §1.821 - §1.825 because sequences have been found in the subject specification. The Notice states that a substitute computer readable form must be submitted as required by 37 C.F.R. §1.825(e).

The Notice states that applicant must provide: 1) an initial or substitute computer readable form (CRF) copy of the "Sequence Listing"; and 2) a statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. §1.821(e) or §1.821(f) or §1.821(g) or §1.825(b) or §1.825(d).

In response, applicants, without conceding the correctness of the Examiner's position but to expedite prosecution of the subject application, enclose a computer diskette containing the sequence listing in computer readable form. Applicants attach hereto, as Exhibit B, a paper copy of the revised computer readable form of the sequence listing. Applicants attach hereto as Exhibit C a Statement in Compliance with 37 C.F.R. §1.821(f) certifying that the computer readable form contains the same information as the paper copy of the sequence listing attached as Exhibit B, and that the sequence listing does not contain any new matter.

In addition, applicants have hereinabove amended the specification to include references to the sequence identifier information (i.e., SEQ ID NO:) as required by 37 C.F.R. §1.821(d). Applicants attach hereto as Exhibit D a version of the amended paragraphs marked-up

to show the changes relative to the previous version thereof pursuant to 37 C.F.R. §1.121(b)(1)(iii). This amendment does not involve any issue of new matter. Therefore, entry of this amendment is respectfully requested.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee is deemed necessary in connection with the filing of this Amendment. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

John P. White
Registration No. 28,678
Alan J. Morrison
Registration No. 37,399
Attorneys for Applicant(s)
Cooper & Dunham, LLP
1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: U.S. Patent and Trademark Office BOX: Sequence, P.O. Box 2327, Arlington, VA 22202

11/18/02

Alan J. Morrison Date
Reg. No. 37,399

EXHIBIT A

UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
www.uspto.gov

APPLICATION NUMBER	FILING/RECEIPT DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
09/750,410	12/28/2000	Gloria C. Li	55672-A-PCT-US/ JPW/AJM/A

CONFIRMATION NO. 6916

John P. White
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, NY 10036

FORMALITIES LETTER

OC000000008821165

Date Mailed: 09/19/2002

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Applicant is given **TWO MONTHS FROM THE DATE OF THIS NOTICE** within which to file the items indicated below to avoid abandonment. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

- This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing", an initial paper or compact disc copy of the "Sequence Listing", as well as an amendment directing its entry into the application. Applicant must also provide a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If applicant desires the sequence listing in the instant application to be identical with that of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF.

For questions regarding compliance to these requirements, please contact:

- **For Rules Interpretation, call (703) 308-4216**
- **To Purchase PatentIn Software, call (703) 306-2600**
- For PatentIn Software Program Help, call (703) 306-4119 or e-mail at patin21help@uspto.gov or patin3help@uspto.gov

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A copy of this notice MUST be returned with the reply.

B12

Customer Service Center
Initial Patent Examination Division (703) 308-1202
PART 2 - COPY TO BE RETURNED WITH RESPONSE

SEQUENCE LISTING

<110> Li, Gloria C.

Burgman, Paul W.J.J.

<120> USES OF DNA-PK

<130> 1747/55672-A-PCT-US

<140> 09/750,410

<141> 2000-12-28

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Dkt. 55672-A-PCT-US/JPW/AJM/MVM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Gloria C. Li et al.

Serial No.: 09/750,410

Filed : December 28, 2000

For : USES OF DNA-PK

1185 Avenue of the Americas
New York, New York 10036
November 19, 2002

U.S. Patent and Trademark Office
BOX: Sequence
P.O. Box 2327
Arlington, VA 22202

Sir:

STATEMENT IN ACCORDANCE WITH 37 C.F.R. §1.821(f)

In accordance with 37 C.F.R. §1.821(f), I hereby certify that the computer readable form containing the nucleic acid and/or amino acid sequences required by 37 C.F.R. §1.821(e) and submitted in connection with the above-identified application, has the same information as the "Sequence Listing," attached as Exhibit B and contains no new matter.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

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Serial No.: 09/750,410
Filed : December 28, 2000

Marked-up Version of Amended Paragraphs

On page 12, lines 10-23:

Figure 14

Nucleotide sequences of Vβ8Dβ2.1Jβ2.6 junctions from the thymus of a 4 week old *Ku70*-/- mouse (SEQ ID NOS: 1-23).

Products corresponding to Vβ8.1, Vβ8.2 or Vβ8.3 rearrangement with Jβ2.6 were cloned and sequenced. TCR Vβ8-Jβ2 joints were amplified by PCR (20, 27, 28) as described (see Fig. 3B). PCR cycling conditions were 94°C for 45", 68°C for 30", and 72°C for 30" (30 cycles). The band corresponding to Vβ8-Jβ2.6 was purified, reamplified for 20 cycles and then subcloned into the pCRII vector (Invitrogen). DNA was extracted from individual colonies and sequenced using the universal T7 and M13 reverse primers. Germline sequences are written in bold case, 'N' and 'P' denote nucleotides not present in the germline sequences.

On page 24, lines 25-32:

The genotype of the mice was first determined by tail PCR analysis which distinguishes endogenous from the targeted *Ku70* allele, and subsequently confirmed by Southern blot analysis. The PCR reaction contained 1 μg genomic DNA; 0.6 μM (each) of primers HO-2: GGGCCAGCTCATTCCTCCACTCATG (SEQ ID NO: 24), HO-3: CCTACAGTGTACCCGGACCTATGCC (SEQ ID NO: 25) and HO-4: CGGAACAGGACTG-GTGGTTGAGCC (SEQ ID NO: 26); 0.2 mM (each) dNTP; 1.5 mM $MgCl_2$ and 2.5 U of Taq polymerase. Cycling conditions were 94°C for 1 min,

On page 25, lines 8-20:

To confirm that the disruption of *Ku70* produces a null mutation, Ku70 protein expression was measured by Western blotting using polyclonal antisera against intact mouse Ku70. The lack of Ku70 was also verified by a Ku-DNA-end binding assay (gel mobility shift analysis). Cell extracts were prepared and gel mobility shift assays were performed as described (22). Equal amounts of cellular protein (50 μg) from *Ku70+/+* (WT), *Ku70+/-*, and *Ku70-/-* mouse embryo fibroblasts were incubated with a ^{32}P-labeled double-stranded oligonucleotide, 5'-GGGCCAAGAATCTTCCAGCAGTTTCGGG-3' (SEQ ID NO: 27). The protein-bound and free oligonucleotides were electrophoretically separated on a 4.5% native polyacrylamide gel. Gel slabs are dried and autoradiographed with Kodak X-Omat film.

On page 27, lines 1-23:

To determine whether a null mutation in *Ku70* affects the recombination of antigen-receptor genes in T and B lymphocytes *in vivo*, we measured the immunoglobulin and T-cell antigen receptor (TCR) rearrangements by PCR. DNA from bone marrow was amplified with primers specific to immunoglobulin D-J_H and V-DJ_H rearrangements, and DNA from thymus was amplified with primers that detect V-DJ_β and D_δ-J_δ-rearrangement (20, 25-28). Oligonucleotides for probes and PCR primers specific to TCR Vβ-Jβ rearrangements and immunoglobulin D-J_H and V-DJ_H rearrangements are as follows. For TCRβ Vβ8-Jβ2 rearrangements (28): Vβ8.1: 5'-GAGGAAAGGT-GACATTGAGC-3' (SEQ ID NO: 28), Jβ2.6: 5'-GCCTGGTGCCGGGACCGAAGTA-3' (SEQ ID NO: 29), Vβ8 probe: 5'-GGGCTG AGGCTG ATCCATTA-3' (SEQ ID NO: 30). For $D_{\delta 2}$-

$J_{\delta 1}$ rearrangement (20, 27): DR6: 5'-TGGCTTGACATGCAGAAAACACCTG-3' (SEQ ID NO: 31), DR53: 5'-TGAATTCCACAG-TCACTTGGCTTC-3' (SEQ ID NO: 32), and DR2 probe: 5'-GACACGTGATACAAAGCCCAGGGAA-3' (SEQ ID NO: 33). For immunoglobulin D-J_H and V-DJ_H rearrangements (26): 5'D: 5'-GTCAAGGGATCTACTACTGTG-3' (SEQ ID NO: 34), V7183: 5'-GAGAGAATTCAGAGACAATC-CCAAGAACACCCTG-3' (SEQ ID NO: 35), VJ558L: 5'-GAGAGAATTCTCCTCCAGCACAG-CCTACATG-3' (SEQ ID NO: 36), J2: 5'-GAGAGAATTCGGCTCCCAATGACCCTTTCTG-3' (SEQ ID NO: 37), 5'IVS: 5'-GTAAGAATGGCCTCTCCAGGT-3' (SEQ ID NO: 38), 3'-IVS: 5'-GACTCAATCACTAAGACA-GCT-3' (SEQ ID NO: 39), and probe: a 6 kb EcoR I fragment covering the J region of mouse IgM.

On page 62, lines 23-34:

The genotypes of the mice were first determined by tail PCR analysis which distinguishes endogenous from the targeted Ku70 allele, and subsequently confirmed by Southern blot analysis. The PCR reaction contained 1 mg genomic DNA; 0.6 mM (each) of primers HO-2: GGGCCAGCTCATTCCTCCACTCATG (SEQ ID NO: 40), HO-3: CCTACAGTGTACCCGGACCTATGCC (SEQ ID NO: 25) and HO-4: CGGAACAGGACTGGTGGTTGAGCC (SEQ ID NO: 41); 0.2 mM (each) dNTP; 1.5 mM $MgCl_2$ and 2.5 U of Taq polymerase. Cycling conditions were 94°C for 1 min, 64°C for 1 min, 72°C for 1 min (30 cycles), followed by an extension at 72°C for 10 min. Primers HO-2 and HO-4 give a product of the targeted

On page 87, lines 17-25:

The genotype of the mice was determined by PCR which distinguishes endogenous from the targeted DNA-PKcs allele. PCR reaction contains 1 μg genomic DNA; 0.6 μM (each) of

primers MD-20: TATCCGGAAGTCGCTTAGCA-TTG (SEQ ID NO: 42); MD-21: AAGACGGTTGAAGTCAGAAGTCC (SEQ ID NO: 43); and POL-8: TTCACATACACC-TTGTCTCCGACG (SEQ ID NO: 44); 0.2 mM(each) dNTP; 1.5 mM $MgCl_2$ and 2.5U of Taq polymerase. Primers MD-20 and MD-21 give a product of wild type allele that is 264 bp; primers MD-20 and Pol-8 yield a product of the targeted allele that is 360 bp.

On page 88, lines 7-19:

For RT-PCR assay, total RNA was prepared from SV40 transformed lung fibroblast cells using Qiagen RNeasy kit (Qiagen Inc., Santa Clarita, CA). After digestion of contaminated genomic DNA by DNase I (Ambion, Austin TX), cDNA synthesis was carried out with the Superscript preamplification system (Gibco BRL, Gaithersburg, MD) according to the included protocol. PCR primers used for RT-PCR were MD-3: ATCAGAAGGTCTAAGGCTGGAAT (SEQ ID NO: 45), MD-5: CGTACGGTGTTGGCTACTGC (SEQ ID NO: 46) for amplification between exon 1 and 4 of DNA-PKcs , MD-28: CACTGAGGGCTT-TCCGCTCTTGT (SEQ ID NO: 47), MD-29: GCTCTTGTGCACGAATGTTGTAG (SEQ ID NO: 48) for PI-3 kinase domain, and GA-5: AGAAGACTGTGGATGGCCCC (SEQ ID NO: 49), GA-3: AGGTCCACCACCC-TGTTGC (SEQ ID NO: 50) for control GAPDH amplification.

On page 89, line 22- page 90, line 12:

T cell antigen receptor (TCR) and immunoglobulin recombination in T and B lymphocytes were measured by amplifying rearranged DNA fragments using PCR. Genomic DNAs were isolated from thymus, spleen and bone marrow (BM) from 4-to 9-week-old DNA-PKcs heterozygous (+/-), homozygous (-/-) mice and SCID

mice. Oligonucleotides for PCR primers and probes are as follow. For TCR_{β} $V_{\beta}8$-$J_{\beta}2$ rearrangement (16), $V_{\beta}8.1$: GAGGAAAGGTGACATTGAGC (SEQ ID NO: 51), $J_{\beta}2.6$: GCCTGGTGCCGGGACCGAAGTA (SEQ ID NO: 29), and $V_{\beta}8$ probe: GGGCTGAGGCTGATCCATTA (SEQ ID NO: 52). For TCR_{δ} $D_{\delta}2$-$J_{\delta}1$ rearrangement, DR6: TGGCTTGACATGCAGAAAACACCTG (SEQ ID NO: 31), DR53: TGAATTCCACAGTCACTTGGGTTC (SEQ ID NO: 53) and DR2 probe: GACACGTGATACAAAGCCCAGGGAA (SEQ ID NO: 33). For TCR_{δ} $D_{\delta}2$-$J_{\delta}1$ signal joint (19), DR21: GTCATATCTTGTCCAGTCAACTTCC (SEQ ID NO: 54), DR162: GATGAGCCAGCTGGATGAGTAACAC (SEQ ID NO: 55), and DR161 probe: GCCCTCTAGCCATGACA TCAGAGC (SEQ ID NO: 56). For immunoglobulin V_H7183-J_H4 rearrangement(19), DR214: CGCGAAGCTTCGT GGAGTCTGGGGGA (SEQ ID NO: 57), DR217: GGGGAATTCCTGAGGAGACGGTGACT (SEQ ID NO: 58), and DR218 probe: ACCCCAGTAGTCCATAGCATAGTAAT (SEQ ID NO: 59). For control GAPDH amplification, same primers were used as RT-PCR experiment. Probe DNA for mouse GAPDH was purchased from Ambion Inc.(Cat.#7330, Austin TX). Amplified PCR products were resolved on 2% of agarose gel in 0.5x TBE, and transferred to Hybond N+ nylon membrane. Using radiolabeled oligonucleotide or DNA probes, PCR products were hybridized and visualized by autoradiography.